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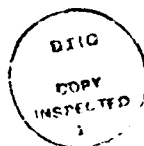University of Utah
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July 1, 1989

MODIFICATION OF QUARTZ SURFACES VIA THIOL-DISULFIDE INTERCHANGE

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ABSTRACT

The present investigation focuses on procedures for silica modification by 3-mercaptopropyltrimethoxysilane (MPS) as studied by attenuated total reflection-fourier transform infrared (ATR-FTIR) and X-ray photoelectron spectroscopies (XPS), along with energy dispersive analysis of X-rays (EDAX). Particular emphasis is given to the accessibility of the surface-attached thiol groups and means for enhancing this accessibility.

From the ATR-FTIR studies, the surface coverage of MPS (Silanization A) and protected-MPS (Silanization B) appears to reach a steady state in about 17 minutes. The elemental composition given by XPS verifies both the presence of MPS on the surface and the stability of the conjugate in alkaline solutions. Narrow region (20eV) scans of the sulfur 2p orbital of the surface-attached MPS reveal the existence of an oxidized form of this element for Silanization A. The possibility of interactions by the thiol group with the surface or other parts of the silane molecule is suggested by XPS and molecular modeling.

An alternative way of producing thiol-containing quartz surfaces is suggested where the mercaptosilane is initially protected by reacting its thiol group with 2,2' dithiodipyridine prior to silanization and subsequently reduced to yield the free thiol group. When modified in this way, the surface shows no evidence of sulfur in the high oxidation state observed for the unprotected silane.

By silver-staining the product of silanization B, it is possible to use both the XPS and SEM/EDAX techniques to assess the fraction of thiol groups which is accessible to a hydrophilic probe. The two analyses exhibit sulfur:silver ratios (S:Ag) which converge to unity for decreasing concentrations of silane. Correlating the different probing depths of the two techniques along with the S:Ag trend indicates that monolayer coverage appears to form in the presence of low concentrations of MPS.

Introduction

Due to their mechanical stability and relative ease of derivatization, surface-modified silicas are commonly used as chromatographic packing materials (1) and as matrix for the attachment of catalytically active substances (2). The desirable optical properties of silica have also made it the material of choice in the growing development of optically based sensors (3). In recent years, much effort has been devoted to the modification of silica through the attachment of biologically active macromolecules, particularly enzymes (4), immunoglobulins (5), and other proteins with a specific affinity toward some compound to be enriched, processed, or detected on the surface.

If the attached protein remains conformationally intact and with its active site exposed, the modified surface will be able to form complexes with binding constants known to reach as high as 10^{15} M^{-1}. Such specifically bound materials are mostly released by drastic changes in the chemical or physical environment of the complex. It is therefore important to select a linking chemistry which leaves the macromolecule intact, while at the same time promoting a stable link between the protein ligand and the silica substrate.

In this context, the attachment of proteins to surfaces via the formation of a disulfide bond appears to be of considerable merit. Proteins naturally contain small amounts of free thiol groups or can be made to contain such groups by the reduction of existing disulfide bridges. Alternatively, thiol groups may be introduced into a protein by reacting a small amount of its free amino groups

with a heterobifunctional reagent, e.g., the commercially available SPDP (6), which was specifically developed for this purpose.

Unlike the amino group, thiol groups are seldom directly involved in the antigen-antibody interaction (7), and are therefore suitable attachment sites for linking antibodies to solid surfaces. In addition, numerous enzymes have been immobilized via their thiol groups to agarose and other hydrophilic matrices with full retention of catalytic activity (8), which further points to these groups as favorable linking sites. A commonly used approach to the thiol-based coupling involves the formation, under mild conditions, of a disulfide bridge between a free thiol on the protein and an activated thiol group on the stationary phase (8).

Thiolation of silica and other mineral surfaces can be accomplished by a silanization procedure involving 3-mercaptopropyltrimethoxysilane (MPS) (9). In a recent survey of the stability of a SPDP-thiolated antibody, which was disulfide-linked to thiolated silica, the conjugate was found to be completely stable over a pH range from 2.0 to 7.5 in the presence of detergents and high concentrations of urea and ethylene glycol (10). In other words, the conjugate was stable under such chemical conditions which may be needed to lower the binding constant for the antibody-antigen complex and accomplish a release of the biospecifically adsorbed moiety.

The choice of silanization procedure has a direct bearing on the composition of the modified substrate. While the desired monolayer

films tend to result from coupling of monofunctional silanes of the type $R-Si(CH_3)_2X$, where X is a halogen or an alkoxygroup (11), their stability is low and the films are readily hydrolyzed even under mild conditions (12). On the other hand, trifunctional silanes tend to form stable films whose thickness far exceeds that of a monolayer (13). Even if smooth and otherwise desirable films were to result from this type of silanization, there is some question as to the reactivity of the introduced functional groups, in this case the thiol groups, for further modification of the surface by disulfide-bridging to proteins or other thiol-containing ligands.

The present study focusses on procedures for silica modification by MPS. Particular emphasis is given to the accessibility of the surface-attached thiol groups and means for enhancing this accessibility.

Materials

Chemicals and materials for the attenuated total reflection-fourier transform infrared (ATR-FTIR) studies were the following: 3-mercaptopropyltrimethoxysilane (MPS) was purchased from Petrarch, cyclohexane (OmniSolv[R]) which was dried over molecular sieve (type 4A, 4-8 mesh from MCB) came from EM Science; cleaning agents included 30% hydrogen peroxide from Malinckrodt along with analytical grades of hydrofluoric acid, ammonium hydroxide, and hydrochloric acid from J.T. Baker. Water for cleaning was from a NANOpure II purification system from Barnstead.

The polycrystalline silicon substrates, part number EE3131, were obtained from Harrick Scientific Corporation. Each ATR-FTIR substrate was a 50 x 20 x 3 mm, 45°, single-pass parallelepiped plate.

Chemicals and materials for the X-ray photoelectron spectroscopy (XPS) and energy dispersive analysis of X-rays (EDAX) experiments were the following: MPS as previously described, 2,2' dithiodipyridine (PDS) and dithiothreitol (DTT) were obtained from Aldrich; cyclohexane, methanol, and acetone of analytical grade, along with crystalline silver nitrate of reagent grade were from EM Science (the cyclohexane was distilled prior to use); filtered ethanol and water, passed through a Milli-Q regent water system by Millipore, were also utilized.

Fused quartz of CO grade came from ESCO Products. Each XPS substrate was cut with a diamond blade followed by polishing to produce chips of size 1 x 1 x 0.1 cm.

Methods

Preparation of Silanization Substrates

The quartz chips were hand-wiped with acetone followed by sonication in acetone (2 x 20 minutes each) and ethanol (2 x 20 minutes each). After drying of the surface, the chips were exposed to a chromic acid bath (70 ml saturated sodium dichromate solution in 9 lbs of concentrated sulfuric acid) at approximately 80°C for 30 minutes. Extensive rinsing with deionized water was followed by baking in a dessicator oven at greater than 100°C for at least 2 hours.

Additional substrate preparations were made prior to the non-aqueous silanizations. Further surface oxidation was provided by a radio frequency glow discharge (RFGD) of oxygen with a Tegal Plasmod. After baking in the dessicator oven, each side of the quartz was exposed for 3 minutes to RFGD of oxygen at an operating pressure of 200μm mercury. Prior to MPS reaction the chips were baked in a vacuum at about 140°C for a minimum of 2.5 hours.

Silanization "A"

This mechanism of silane treatment was adapted from one used for quartz particles (14). The cyclohexane solvent was distilled and stored over $CaSO_4$ to remove unwanted moisture. All glassware was prerinsed with the solvent before the reaction started. MPS with a concentration of 10% (v/v) was mixed with cyclohexane at room temperature in a closed chamber funnel. The funnel was purged with prepurified (>99.95%) nitrogen gas. Quartz chips were placed in a 3-necked round flask which had a thermometer, a stopper, and the MPS solution-containing funnel in the openings. Using a heating mantel controlled by a Staco variable autotransformer to increase the temperature to about 50-60°C, the quartz was exposed to the MPS for 2 hours in the nitrogen atmosphere. The chips were subsequently rinsed 5 times with cyclohexane to remove nonbinding MPS and then 3 times with water for surface-crosslinking of the coupling agent. The last step was a vacuum baking of the treated substrate at approximately 120°C for greater than 1 hour.

Silanization "B"

This mechanism parallels that of Silanization A with the exception of the MPS being in an oxidized disulfide form before substrate treatment. The MPS reagent was saturated with PDS at room temperature for 10-20 minutes to produce a disulfide-linked pyridine modification. The reacted silane, whose presence was indicated by a yellowing of the solution, was subsequently purified by vacuum distillation. Since this version of MPS should be less vulnerable to atmospheric oxidation, it is termed protected-MPS. This form of MPS was then reacted with the quartz as described for Silanization A. The formula below shows the chemical structure of the organosilane in its disulfide-protected form.

$HS(CH_2)_3Si(OCH_3)_3$ + (PDS) ⟶ (protected) + (pyridine)

MPS   PDS (excess)   Protected Form
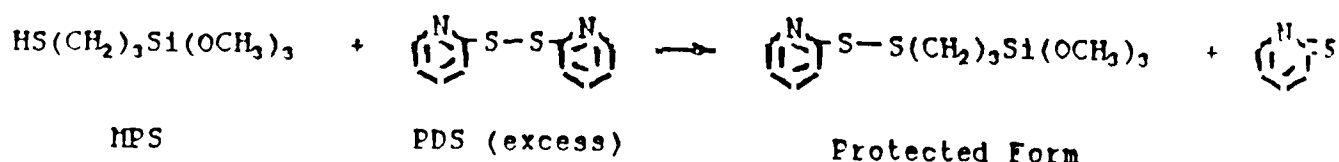
In later experiments with this pathway, the concentration effects of protected-MPS were studied. Substrates were reacted with 0.1, 0.5, 1.0, 5.0, and 10% (v/v) of protected-MPS.

Activated-Polyethylene oxide (PEO) Coupling Procedures

Following MPS treatment, the quartz samples were placed in separate 12x75 mm borosilicate culture tubes (Fisher Scientific). Reduction with DTT to remove the protecting group and washing, as previously described, immediately preceded the reaction with activated-PEO. The initial experiments used $2 \times 10^{-3}M$ concentrations of activated-PEO with a molecular weight of 1000 in

TRIS buffer of pH=8.6. The reaction mixtures were maintained overnight at room temperature and will be referred to as A + PEO 1000* and B + PEO 1000* to represent data for silanizations A and B with activated-PEO MW=1000, respectively.

Silver-Staining Procedures

Accessibility of sulfhydryl groups on the surface was probed by chemical derivatization. Solutions of 1 mM silver nitrate were reacted overnight at room temperature with the silanized samples. In the Silanization B cases, the treated substrate was first reduced with 2.5×10^{-4}M DTT for 20 minutes at room temperature followed by 3 water rinses and immediately reacted with the silver solution. The silver-coated substrates were rinsed with water prior to further analysis.

Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR)

The ATR-FTIR technique used for determining in situ kinetics has been described elsewhere (15). The substrate cleaning method and a description of the ATR-FTIR system can be found in reference (15).

The solution used to fill the ATR cell consisted of 10% (v/v) silane in dried cyclohexane. Duplicate experiments were run to determine the binding kinetics of both MPS and protected-MPS.

Ellipsometry measurements were made after the oxide layer was grown on the silicon substrate and again after the silane was bound to the oxide layer to determine film thicknesses. The analyses were run on a Gaertner model L117 ellipsometer. Data were processed by the method of McCrackin et al (17).

Molecular Modeling

A simple molecular model of the two silanes, MPS and protected-MPS, was investigated using the Quantum chemistry Interactive Program Utility (QUIPU)(18), which ties together a collection of molecular modeling tools. The following calculations within QUIPU were performed: SKETCH, MODIFY (standard), CFM, and Alinger's MM2 (optimize). Due to difficulties in obtaining a consistent set of force constants and bond information for silicon, silicon atoms were approximated by substituting carbon atoms. A substitution of silicon atom size for carbon atom size was made before PLUTOP79 was used to plot the data on a laser printer. The large size of the molecules tested, the inexact silicon atom calculation, and the lack of information on interactions between molecules are recognized as considerable sources of error within these calculations.

X-ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) was carried out using a Hewlett-Packard 5950B electron spectrometer equipped with a monochromatic Al Kal,2 radiation source at 1487 eV and 400 watt power at the anode. Water-hydrolyzed MPS films were prepared at room temperature and allowed to dry on a copper substrate. XPS was then utilized to assess the elemental composition of the solid, water-mixed MPS layer. Narrow scans (20eV binding energy range) of the carbon 1s, sulfur 2p, and oxygen 1s orbitals were used for quantitation.

Quartz chips were cleaned and silane-treated prior to examination by XPS for the purpose of determining the elemental compositions of the 2 silanization pathways. In addition to the scans taken for the MPS film, the nitrogen 1s signal was monitored. Control data were taken for cleaned plus oxidized and cyclohexane solvent exposed chips.

The stability of the silane-treated quartz was assessed by exposure of samples produced by method A to a 0.2M TRIS buffer solution pH=8.6 for various times.

## Scanning Electron Microscopy/Energy Dispersive Analysis of X-rays (SEM/EDAX)

SEM in combination with EDAX was performed on the silver-coated substrates of Silanization B. Two samples were prepared for each concentration of protected-MPS. Prior to placement into the high vacuum chamber of the microscope, each specimen was sputtered with carbon to lessen any surface charging effects. The microscope was a JEOL JEM-200CX SEM with a Kevex EDX attachment. The elemental data were obtained from sample spots with a diameter of about 200Å.

Results and Discussion

3-Mercaptotrimethoxysilane (MPS) is a commercially available, stable silane. Like other trialkoxy-silanes, MPS is known to readily polymerize in aqueous solution; the silanization of silica surfaces with these reagents in the presence of water is therefore frequently observed (11) to yield thick and uneven surface coatings. Since the goal in this study was to generate smooth,

stable substrates with a high density of reactive thiol groups, a coupling procedure was adopted in which the quartz substrate was allowed to react with the silane in freshly distilled, dry cyclohexane. Similar conditions have been shown by previous authors (14) to yield relatively thin and even surface coatings of good stability.

Once thiolated, these surfaces were to be derivatized in a thiol-disulfide interchange reaction with a thiol-containing macromolecule whose -SH group had been previously activated to facilitate coupling. The overall reaction, schematically indicated below, has been studied in detail by Brocklehurst, Carlson and others (8,19-21):

Substrate-SH + 2-pyridyl-S-S-Ligand →

Substrate-S-S-Ligand + 2-Thiopyridone
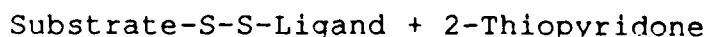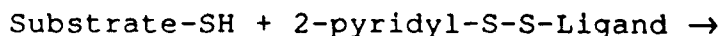
Rate of Silanization

The choice of reaction time for the silanization step was based on a spectroscopic study in which the enrichment of MPS at the quartz-cyclohexane interface was measured at 3 minute intervals using ATR-FTIR. This technique was recently developed (15), and applied to a model system where diphenylchlorosilane was allowed to react with silanol groups on the surface of the quartz prism confining the IR beam. The time dependent interfacial enrichment of MPS is exemplified by the ATR-FTIR spectra in Figure 1, which demonstrate the change in intensity of peaks in the C-H stretching region with time. Figure 2 shows the changes in intensity which occur for the major peak in these spectra during a period of one

hour. Variations between duplicate experiments were minor, as seen in the figure. From these studies, the surface coverage appears to reach a steady state in about 17 minutes.

As discussed in reference (15), the depth of penetration of the evanescent wave past the edge of the substrate changes almost linearly between about 4000 and 2000 cm^{-1} for organic solvents without absorption bands in that wavelength range. However, bands are found in the 3000 cm^{-1} region for the solvent cyclohexane, so the depth of penetration here is slightly nonlinear. Extrapolating from values found for the index of refraction of cyclopentane in the infrared range (16), penetration depths of nearly 3000 Å at 3000 cm^{-1} are estimated for cyclohexane. This means that if silane polymer formation continued beyond that thickness, it would not further affect the ATR-FTIR kinetic data. Thus, the flat portion of the rate curve in Figure 2 may not be an indication of a completed reaction, but may rather be an artifact due to the build-up of a silane film beyond the depth of penetration of the surface evanescent wave.

After one hour, the MPS binding reaction was interrupted. The modified surface was then carefully rinsed with clean solvent and examined by ellipsometry, which indicated that the MPS film had grown to a thickness of about 100 Å. At this point, it is impossible to say whether the build-up of the film came to a halt at 100 Å, which is well within the measuring range of the ATR-FTIR technique, or if layers deposited beyond the depth of penetration had been removed in the rinsing process.

The peaks shown in Figure 1 are negative in character, meaning that with time some species are leaving the surface. Figure 3 shows the C-H stretching range of the silane by itself. Spectra for the solvent, cyclohexane, are available elsewhere (22). A comparison of these spectra shows that both cyclohexane and MPS have peaks in the same part of the C-H stretching region; however, neither by itself appears to have all the features of the spectrum in Figure 1. Since the peaks are so similarly placed, no effort was made here to determine peak assignments for the spectra collected. It is possible that the peaks from the solvent and that from the silane are both becoming negative at the same time. As the surface film polymerizes, excess silane molecules may have to leave the surface region along with solvent molecules. The major peak for both substances in this region grows to a maximum height over the same period. However, no additional information could be gained by comparing the rates of change of other spectral features.

Properties of MPS-Modified Quartz

The product of this modification procedure was analyzed by XPS to verify the presence of MPS on the surface. From the observed atomic percentages of C(1s): S(2p): Si(2p): O(1s), which were $(34.7 \pm 6.7) : (1.3 \pm 0.4) : (28.5 \pm 2.7) : (35.5 \pm 5.9)$ respectively, it is obvious that surface modification had occurred. The large amount of silicon relative to sulfur is an indication that the silane layer is either thin or patchy, and that much of the quartz substrate is being probed by the X-ray

beam. If the depth of analysis, which should be less than 100 Å, had been exceeded by the thickness of the silane film, the stoichiometry of the MPS molecule would have resulted in comparable atomic percentages for sulfur and silicon.

Since the modified quartz was to undergo a thiol-disulfide interchange reaction, which is promoted by alkaline conditions (20), it was important to establish the stability of the silane film under basic pH. A set of 21 MPS treated silica chips was exposed to a 0.2M TRIS buffer of pH 8.6. Samples in triplicate were removed at different times and analyzed for changes in sulfur by XPS. At no time during 1600 minutes of exposure to the alkaline buffer was the sulfur content reduced by more than 10% in comparison with the composition prior to exposure. The modified substrate was thus considered stable enough to undergo even slow interchange reactions without degradation.

Thiolated polyethyleneoxide (PEO(SH)$_2$) with a molecular weight of 1000 had been prepared for use as a spacer arm in linking proteins to the thiolated substrate (23). In the present study, our goal was the surface attachment of PDS-activated PEO(SH)$_2$ in high yield. Remarkably, an attempt at coupling the activated PEO for a period of 48 hours at room temperature proved completely without result, as XPS failed to demonstrate the presence of ether carbon on the surface of samples from this coupling study (see Table 1). A similar lack of reactivity of surface-linked MPS had also been observed elsewhere(24,25).

Table 1 compares the carbon 1S peak fitting ratios for samples from the unmodified and modified MPS treatments upon reaction with

activated poly(ethylene oxides) (PEO 1000*). The gaussian peak-fitted data suggested the presence of ether-bonded carbon (C-O-C), with its chemical shift of approximately 1.8eV, for the protected MPS but not for the surfaces treated with unprotected MPS. The alkyl carbons (C-C) still comprised the majority of the Cls peak. This indicated limited but greater coupling of PEO 1000* onto the protected MPS-treated substrates than for the unprotected case.

It was noted that both the PEO-treated samples and samples never exposed to this reactant displayed a sulfur peak in XPS which corresponded to a higher state of oxidation than that of the thiol group. A typical segment in the sulfur region of the XPS spectrum (150 - 170 eV) is shown in Figure 4a. Here, the major S(2p) peak, characteristic of the thiol sulfur, is accompanied by a somewhat less intense peak whose position is shifted upfield by about 4 eV. As can be seen in Figure 4b this peak is absent from the XPS spectrum of pure MPS, which had been deposited on a copper substrate and subsequently hydrolyzed in the presence of water. It is apparent that the existence of this form of oxidized sulfur was somehow related to the quartz attachment of the silane.

Figure 5a, which represents a molecular modeling of MPS, gives support for the theory that the folding of the MPS propyl chain may permit the thiol group to interact with available hydroxyls either on the hydrolyzed silane or on the silica substrate. This theory is further supported by proposed structures for similar, but amine-containing silanes(12). In addition, a hydrogen-bonded surface-silane ring configuration has been previously proposed (25) for the MPS attached to a silica substrate. The modeling

observations which show the folding tendencies of the MPS propyl chain correlate with existing hypotheses (24,25).

Coupling with Protected Thiol Groups

In order to enhance the availability of surface-bound thiol groups, Olofsson et al. (24) designed a proprietary method for protecting the MPS thiol prior to the silanization reaction. Once the attachment of the modified MPS had been completed, and the thiol freed from its protection, the surface appeared available for thiol-disulfide interchange. This observation suggested to us the option of reacting the MPS thiol with dipyridyldisulfide (PDS), in a manner analogous with the thiol activation described by Brocklehurst (19) and Carlson (20). This reaction, which occurs rapidly and with high yield, is outlined in the Methods section under the heading of Silanization B. A molecular graphics representation of the PDS-protected MPS is shown in Figure 5b. From this representation, it appears that the bulky PDS group prevents the propyl chain from folding back towards the interface, instead leaving it in a position more accessible to the bulk solution.

As in the case of the unprotected silane, the rate of accumulation of protected MPS at the quartz interface was analyzed by ATR-FTIR. From Figure 6 we conclude that the protection has no observable effect on this reaction rate, since the shapes of the rate curves in Figures 2 and 6 are virtually identical, with an apparent steady state being reached after 17 minutes in both cases. This finding suggests that silanizations involving the

protected-MPS (Silanization B) can be carried out under identical conditions to those used for the unprotected form (Silanization A). An XPS analysis of the product of Silanization B is shown in Figure 7. The sulfur peak is formed at 159.12 eV which is characteristic for disulfides. Unlike the product of Silanization A, this surface showed no evidence of sulfur present in the higher (162.96 eV) oxidation state.

After completion of the type "B" silanization, the MPS protecting groups were removed through reduction with 0.1M dithiothreitol (DTT). The substrate was then thoroughly rinsed with distilled water, followed by 0.2M TRIS buffer of pH 8.6, in preparation for the thiol-disulfide interchange reaction with activated PEO-(SH)$_2$, as described above. This time, the substrate did indeed accept the ligand, as can be seen from the XPS detected surface-bound ether carbons reported in Table 1. However, since each dithiolated PEO molecule brings to the surface about 45 ether carbons and 2 sulfurs, the observed 5-fold excess in the latter would indicate a very modest coupling yield of about one PEO molecule per 220 surface-bound thiols.

Assessment of Accessible Thiols

The low coupling yield for PEO could be explained using steric arguments, since the size of the molecule (radius of gyration 7.5 Å in aqueous solution (26)) will affect both the rate of attachment and the close-packing of ligands on the surface. Therefore, it is clearly preferable to examine the attachment of a smaller, water soluble probe to quantify the accessible thiols on

the quartz surface. Our choice of the Ag^+ ion as such a probe was based on two conditions: a) the high affinity between the silver ion and the thiol group (27), and b) the high electron density and high Scofield cross-section (28) of this heavy metal ion. The latter properties will facilitate a more informative analysis of the stained surface by SEM / EDAX and XPS, respectively.

Chemical coupling of silver to the silanized quartz also provides an alternate approach to analyzing the functionality of MPS with XPS. The derivatizing reagent, $AgNO_3$, contains a unique elemental (silver) tag which is readily identified. An increased Scofield cross section for silver compared to sulfur tends to increase the sensitivity of XPS. The lack of an observable Ag3d signal for cleaned substrates exposed to $AgNO_3$ suggests that virtually no adsorption of silver occurs on the untreated quartz. In contrast, a strong silver peak emerges after reaction of the derivatizing reagent with MPS-treated samples. These results are an indication that coupling of silver occurs with the sulfhydryl group.

Scanning Electron Microscopy (SEM) of the silver stained, MPS-coated substrate revealed a rough surface, seemingly full of submicron sized particles. The surface density of these particles could be significantly reduced by performing the silanization reaction at MPS concentrations below the 10% (v/v) used initially. Indeed, a series of derivatizations performed at MPS concentrations of 10%, 5%, 1%, 0.5%, and 0.1% respectively, showed a steadily decreasing amount of surface bound particles.

The circular area probed by the electron beam in these SEM experiments has a diameter of approximately 200 Å. When one of the surface particles was visibly within the probe area, the amounts of sulphur and silver reached levels detectable by the dispersive X-ray fluorescence (EDAX) analyzer attached to the microscope. Table 2 lists the S:Ag atomic ratios recorded by SEM/EDAX for the different surfaces. These same silver-stained substrates were also examined by XPS, which is more surface sensitive than SEM/EDAX. Since the XPS analysis covers a larger area, the observed atomic ratios of sulfur to silver listed in Table 2 are average values over 5mm^2.

If all surface attached thiol groups were available to react with a water soluble probe such as Ag$^+$, the atomic ratio of sulfur to probe would be unity. The data in Table 2 are an indication that silanization using dilute solutions of protected MPS , followed by a reduction step, does indeed produce a large fraction of reactive thiols. The quantitative differences observed between the SEM/EDAX and XPS-derived S:Ag ratios are seen to be the largest for those substrates produced with the most concentrated silane solutions. Here, the relatively depth-probing SEM/EDAX indicates a sulfur to silver ratio of 9:1, suggesting that only slightly more than 10% of the surface thiols are accessible to a hydrophilic reagent. By contrast, the more surface-probing XPS technique indicates that 75% of all measured thiols are accessible. However, surfaces produced with lower concentration of MPS give progressively better agreement between the two techniques, as both give sulfur:silver ratios approaching unity.

This agreement is an indication that the surface film of MPS is getting thinner, which in turn ensures virtually complete access to the surface bound thiols.

Conclusions

This work indicates that stable derivatives are formed as MPS is allowed to react with silica in nonaqueous solvents. The initial attachment of the silane reaches an apparent steady state in about seventeen minutes and, after washing and curing, the conjugate can withstand exposures to mildly alkaline conditions (pH 8.6) for periods in excess of 24 hours without a significant loss in surface-bound sulfur. However, this mode of surface thiolation yields a product with a low content of free or accessible thiol groups. An XPS analysis of the product indicates that the sulfur is present on the surface in a higher state of oxidation than in the mercaptosilane itself.

An alternative way of producing thiol-containing quartz surfaces is suggested, and shown to result in a greater amount of accessible thiol on the surface. In this approach, the mercaptosilane is protected by reacting its thiol group with PDS prior to silanization. The rate of the silanization reaction appears to be unaffected by the presence of the protecting group, which is easily reduced off once the attachment is completed. When modified in this way, the surface shows no evidence of sulfur in the high oxidation state observed for the unprotected silane.

By silver-staining the product, it is possible to use the XPS and SEM/EDAX techniques to assess that fraction of thiol groups

which is accessible to a hydrophilic probe. Since the two techniques tend to probe the surface to different depths, the convergence of the two analyses to an S:Ag ratio of unity, when progressively lower concentrations of silane are used to modify the silica substrate, indicates that monolayer coverage appears to form in the presence of low concentrations of MPS.
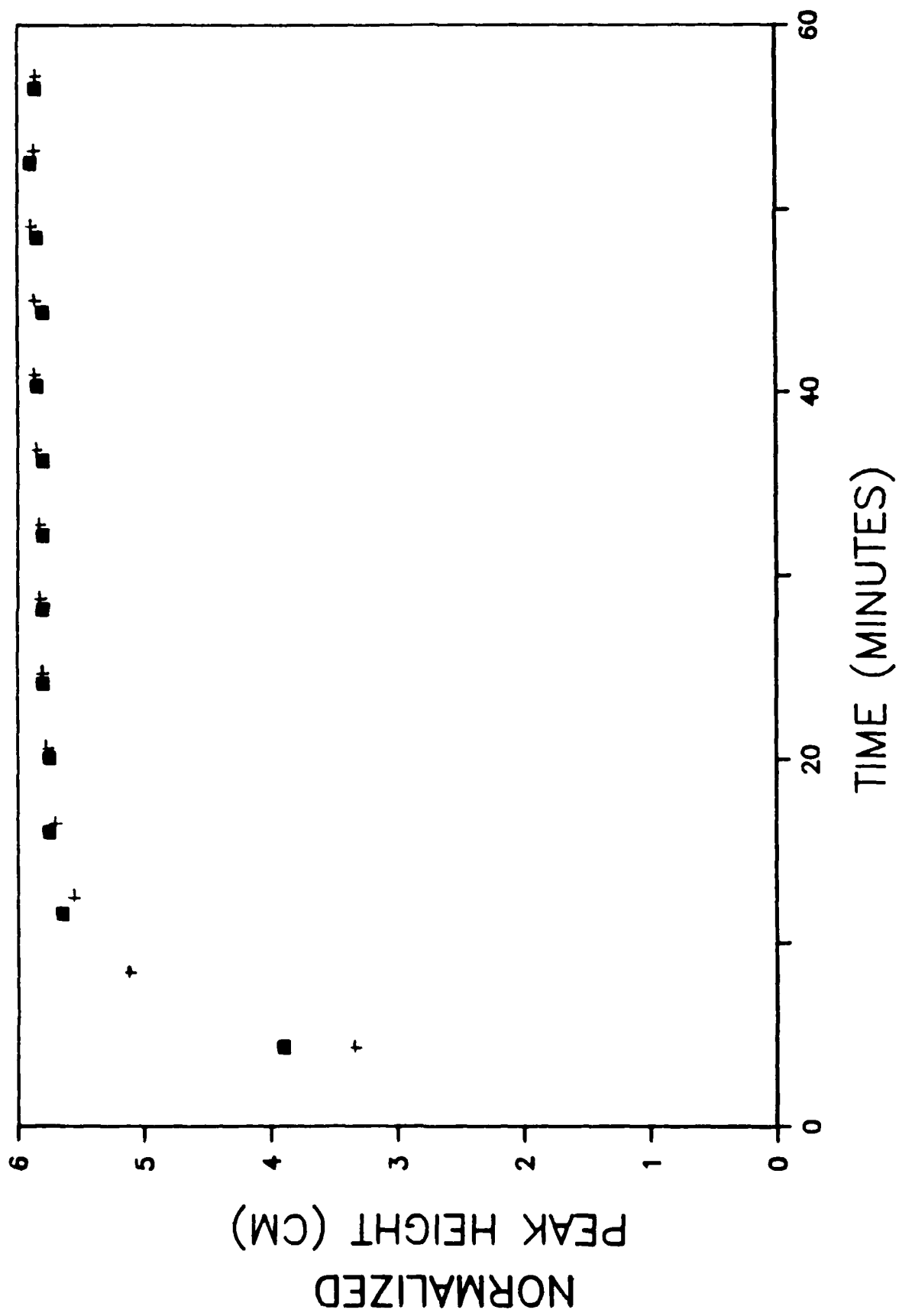
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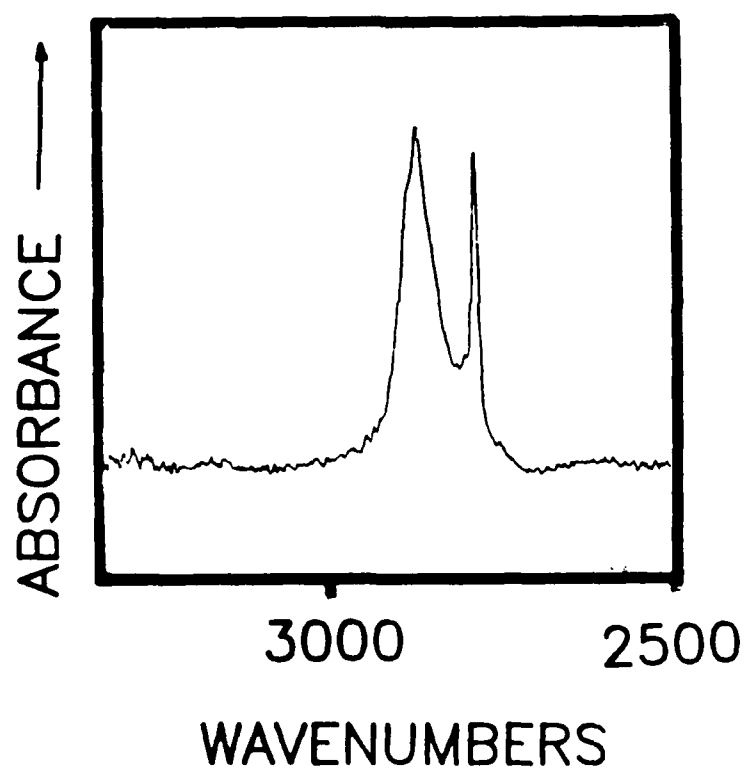16. Goplen, T.G., Cameron, D.G., Jones, R.N. *Appl. Spec.* **1980**, *34*, 657.

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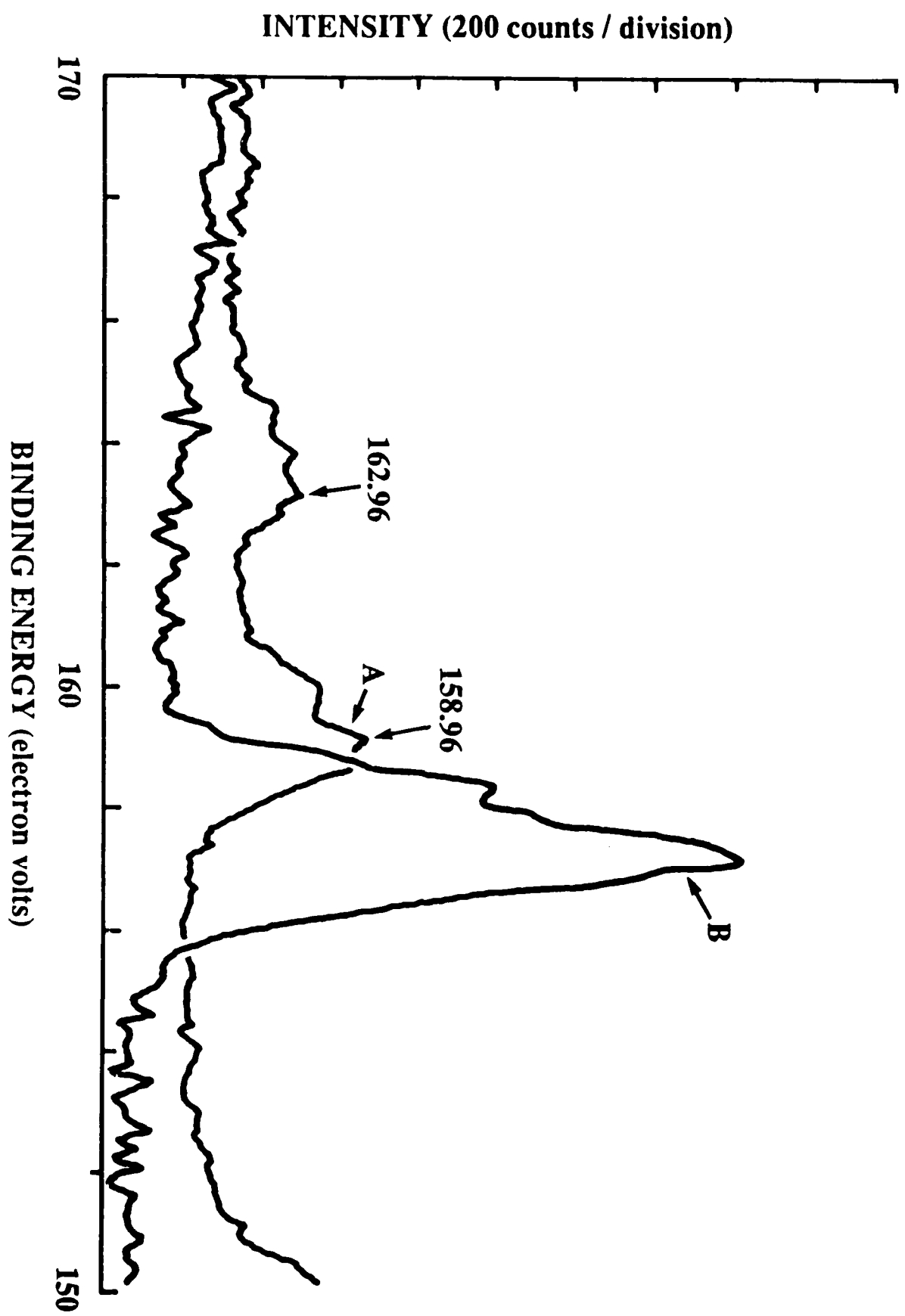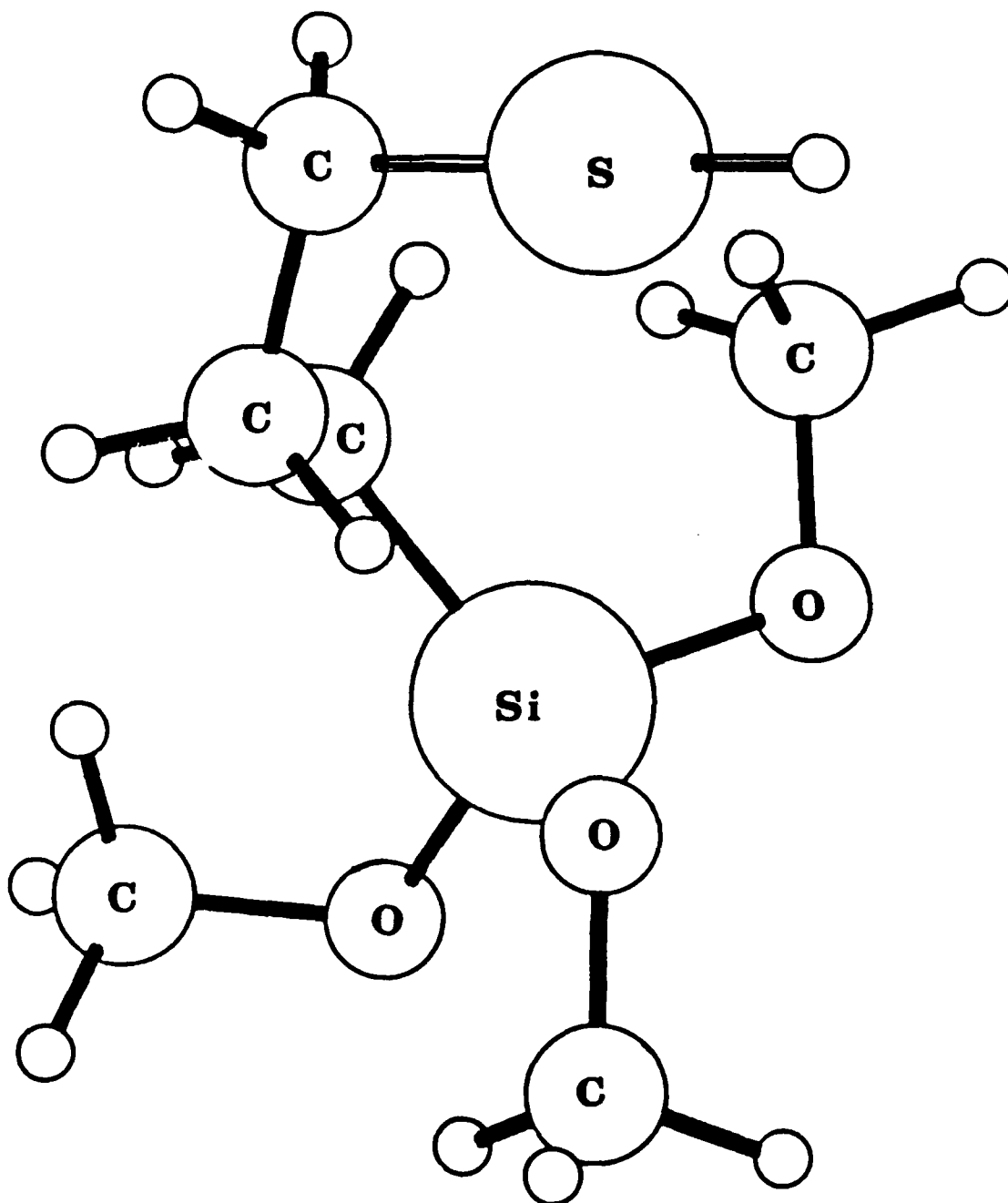
Figure Legends

1. ATR-FTIR difference spectra of 10% by volume MPS in cyclohexane binding on oxidized silicon. The background used was the initial spectrum collected when the cell was filled with the silane solution. The spectra shown were collected after A) 4.2, B) 8.3, and C) 12.4 minutes.

2. Normalized peak height vs time for the largest negative peak in the difference spectrum for 10% (v/v) MPS in cyclohexane binding to oxidized silicon. The + and symbols represent different runs of the same experiment.

3. Difference spectrum showing 5% MPS in carbon tetrachloride. The background used was an empty cell.

4. XPS spectra of the sulfur 2p orbital for A) unprotected MPS coupled to quartz and B) hydrolyzed MPS on copper as a control. Notice the emergence of a peak shifted by 4 eV from the main signal response of spectrum A.

5. QUIPU's PLUTOP79 plot of A) MPS and B) protected-MPS.

6. Normalized peak height vs time for the largest negative peak in the difference spectrum for 10% (v/v) ofprotected-MPS in cyclohexane binding to oxidized silicon. The + and symbols represent different runs of the same experiment.

7. XPS spectrum of the sulfur 2p orbital for protected-MPS coupled to quartz. Notice the disappearance of the shifted peak (162.96 eV) shown in Figure 4.
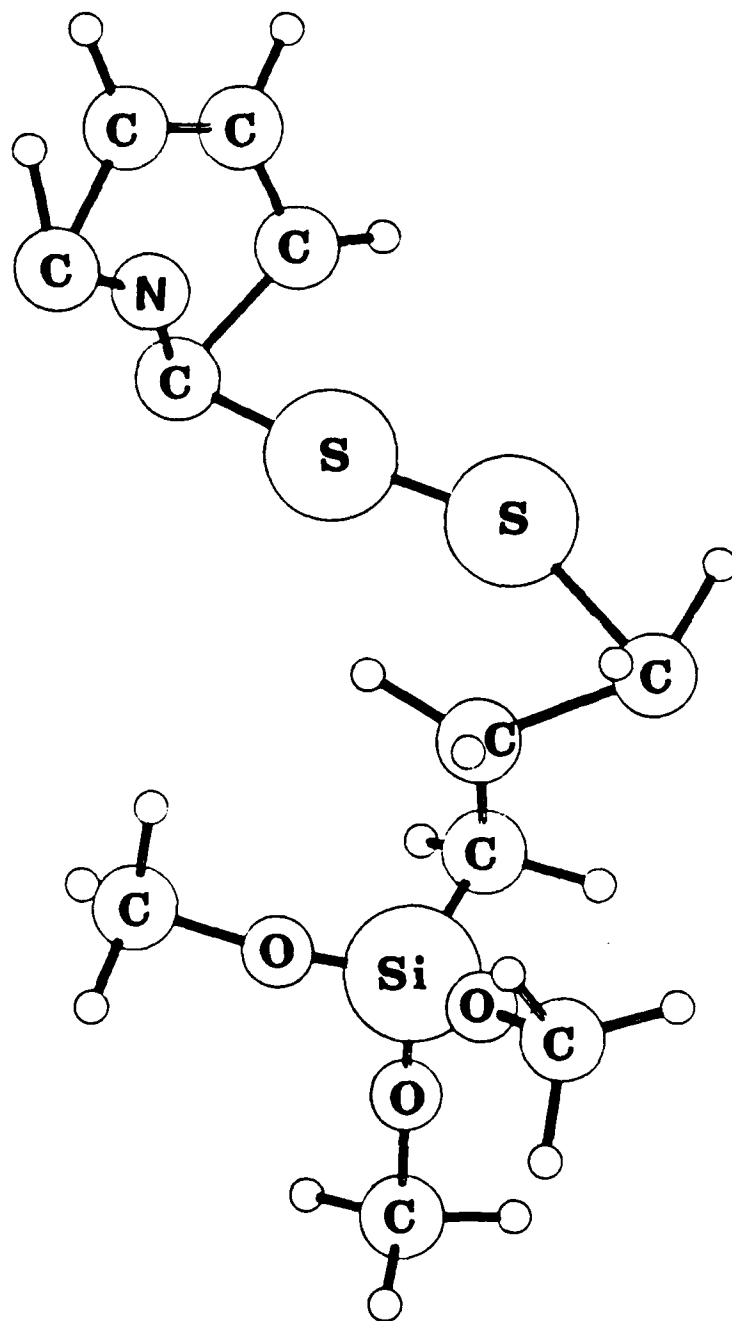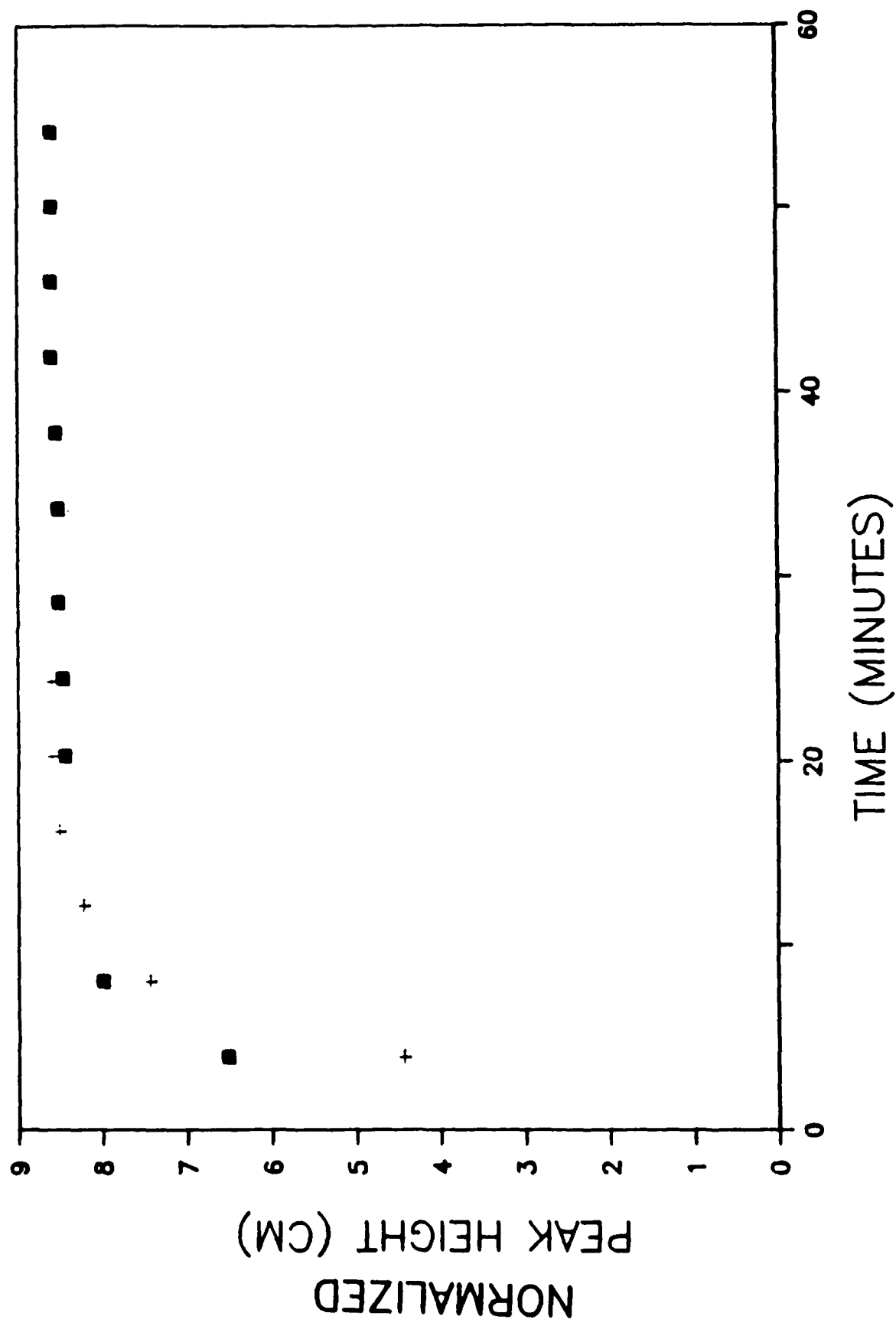
ABSORBANCE

A

B

C

3000   2500

WAVENUMBERS

Yee, Et Al.   Figure 1A,B, & C

NORMALIZED PEAK HEIGHT (CM)

TIME (MINUTES)

— Yee, Et Al. Figure 2

ABSORBANCE

3000   2500

WAVENUMBERS

INTENSITY (200 counts / division)

BINDING ENERGY (electron volts)

162.96

158.96

A

B

Yee, Et Al. Figure 4A&B

NORMALIZED
PEAK HEIGHT (CM)

TIME (MINUTES)

— Yee, Et Al. Figure 6

INTENSITY (200 counts / division)

BINDING ENERGY (electron volts)

159.12

170   160   150

— Yee, Et Al.   Figure 7

Table 1

Comparison of Carbon 1s Peak-fitting Ratios

Surface	C-C[a]	C-S[b]	C-O-C[c]
A[d] + PEO 1000*	4.1 ± 0.4	1	---
B[e]	4.5 ± 0.7	1	---
B[e] + PEO 1000*	3.8 ± 0.2	1	0.2 ± 0.03

[a]alkyl type carbon bond
[b]carbon-sulfur type bond
[c]ether type carbon bond
[d]product of Silanization A
[e]product of Silanization B
*mercapto-activated poly(ethylene oxide) MW=1000
reported errors represent analyses of sample triplicates

Table 2

Comparison of Sulfur:Silver Ratios of Atomic Percentages Determined by EDAX and XPS[a]

MPS[b]	EDAX S:Ag	XPS S:Ag
10%[c]	9.15	1.29
5%	2.6 ± 1.2	1.7 ± 0.8
1%	2.1 ± 0.1	1.8 ± 0.7
0.5%	3.9 ± 3.5	1.0 ± 0.4
0.1%	1.8 ± 0.1	1.5 ± 0.6

[a]using Silanization B
[b]concentration of silane (v/v)
[c]one trial reported
reported errors represent analyses of sample duplicates